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14. ABSTRACT
We show that an ultrafast laser plasma x-ray source made by focusing a fs laser onto a liquid mercury target can be used for phase contrast imaging of a murine liver in vitro. The x-ray spectrum emitted at 5-kHz repetition rate has characteristics of a 2.5-W, 30-kV microfocus x-ray tube with a tungsten anode. The phase contrast images of the liver show the venous network with approximately 20-µm spatial resolution without the use of a phase contrast agent. The method permits taking tomography images that can be viewed as orthogonal cross sections. Second, we report a new preparative method for providing contrast through reduction in electron density that is uniquely suited for in-line, X-ray phase contrast imaging. The method, which results in an air filled vasculature, makes possible visualization of the smallest microvessels in an excised murine liver.

15. SUBJECT TERMS
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Introduction

Laser-plasma source for phase contrast imaging
and
Preparative method for ex vivo liver imaging

Since laser plasma sources are generated by optical radiation focused to a diffraction limited spot size, the spatial coherence of the resulting x-ray burst can be quite high. This high coherence property can be used for in-line holographic imaging, enabling the measurement of x-ray phase shifts induced by an object in the x-ray imaging beam path. The optical in-line arrangement of x-ray source, object, and detector resembles that of conventional radiography. However, phase contrast imaging is fundamentally different from conventional x-ray shadowgraphy because the mechanism of image formation does not rely on differential absorption by the object[1-3]; rather, x-ray beams undergo differential phase shifts in passing through the material and subsequently interfere constructively or destructively at the x-ray camera or film. Materials in an irradiated body are distinguished by their different indices of refraction rather than their purely absorptive properties[4-7].

In Propagation-based Differential Phase Contrast Imaging (PDPCI), x-ray waves passing through different but adjacent volumes of the sample interfere. The image contrast caused by x-ray phase shifts can be up to a factor 1000 larger than that for x-ray absorption contrast. Phase sensitive x-ray imaging, has been carried out using synchrotrons and x-ray tubes. However, it is only recently that laser plasma sources have been used for phase-sensitive imaging applications of static objects[8-10]. Here, we show that a laser plasma source can be used to obtain high resolution images of an excised mouse liver.

We also describe a sample preparation technique that is uniquely suited to phase contrast imaging. Vascular growth and degeneration play an important role in the dynamics of numerous human pathologies. For example, it is now known that many types of cancer signal neovascularization from nearby blood vessels allowing the tumor access to nutrients essential for growth and metastasis. Aside from cancer malignancy, blood

vessel birth and death also plays an important role in wound healing, embryonic development, and atherosclerosis. As a result of the role that vascular dynamics plays in pathology, vascular imaging methods have become of great importance not only for observing vascular proliferation and degeneration, but also as a method for determining the efficacy of drugs aimed at suppression or promotion of neovascularization.

There are several techniques for visualization of vasculature which include vascular casting and high resolution x-ray imaging. In the former, a cast of the vasculature is made, which, when viewed with a microscope, has been shown to provide a powerful means for visualizing the finest features of vascular networks. However, these techniques destroy the tissue sample and do not permit the observation of dynamic processes. When used with a contrast agent, newly developed, non-invasive, high-resolution x-ray imaging systems have permitted imaging of biological tissue on the micron and nanometer scale. For imaging the vascular structure of soft tissue, radiological contrast is typically produced through the use of injected contrast agents, which provide increased x-ray absorption. In spite of their relative simplicity, these procedures using contrast agents have not yet produced images with micrometer resolution of liver microvasculature.

Here, we report on sample preparation for phase contrast imaging of the liver microvasculature in excised murine livers. Since blood vessels collapse after excision, we developed a preparation method that re-opens the venous system of the liver. This re-opening process was visualized in time with x-ray, phase contrast imaging. We show that as a result of re-opening the blood vessels and their subsequent filling with air, large density gradients are formed at the walls of the vessels that yield images having both high resolution and remarkable contrast. Experiments are reported where the re-opening of the vessels is recorded in time as the liver dehydrates.

Body

The laser plasma source used here is based on a flowing jet of liquid mercury onto which 40-fs, 800-nm, 3-mJ laser pulses are focused with a repetition rate of 5 kHz. A prepulse that precedes the main pulse by 50ps with an amplitude of 4% of the main pulse is generated to improve the x-ray generation efficiency. The x-radiation was detected by direct exposure of the CCD-chip of a liquid-nitrogen cooled x-ray camera. The emitted x-ray flux in the spectral range useful for imaging, 9 – 30 keV photon energy, is 2 x 10^{11} ph / (s 4π sr). The laser plasma source emission, which is the largest continuum-flux ever measured from a liquid target, is similar to that of a tube operated at 30 kV acceleration voltage and 2.5 W power. The horizontal and vertical dimensions of the source were measured using the knife-edge method as 38 µm x 13 µm (fwhm).

The source's imaging performance in illustrated in Fig. 1 where an image of a mouse leg is shown. In this image, absorption contributes to the image contrast and additional phase contrast features are visible at the interfaces of soft tissues as slight contrast enhancements. The image sequence in Fig. 2 shows an image of the veins in a mouse liver that was excised from an euthanized mouse, fixed in paraformaldehyde and subsequently dried. The vascular tree is clearly visible in the x-ray image. Contrast agent injections into the portal vein of another mouse liver verified that the veins are imaged and not the arteries or bile ducts. To our knowledge, these images exhibit the highest resolution and contrast of the vasculature of any organ taken with any laboratory x-ray source to date. The smallest vessels have a diameter of about 20 µm, just a little larger than the diameter of red blood cells (6.6 - 7.5 µm). For illustration, scaled pictures of erythrocytes are pasted into the rightmost image. The vessels are phase-contrast highlighted which enhances their visibility. The images were measured with a magnification of 3, a source-to-CCD camera (Princeton Instruments, PI-SCX, 4k x 4k, 1:1 fiber-coupled) distance of 1.5 m and an exposure time of 3 min. As shown in Fig. 1, samples absorb all radiation below 9 keV resulting in a transmission of about 30% of the incident radiation, which represents significant beam hardening.

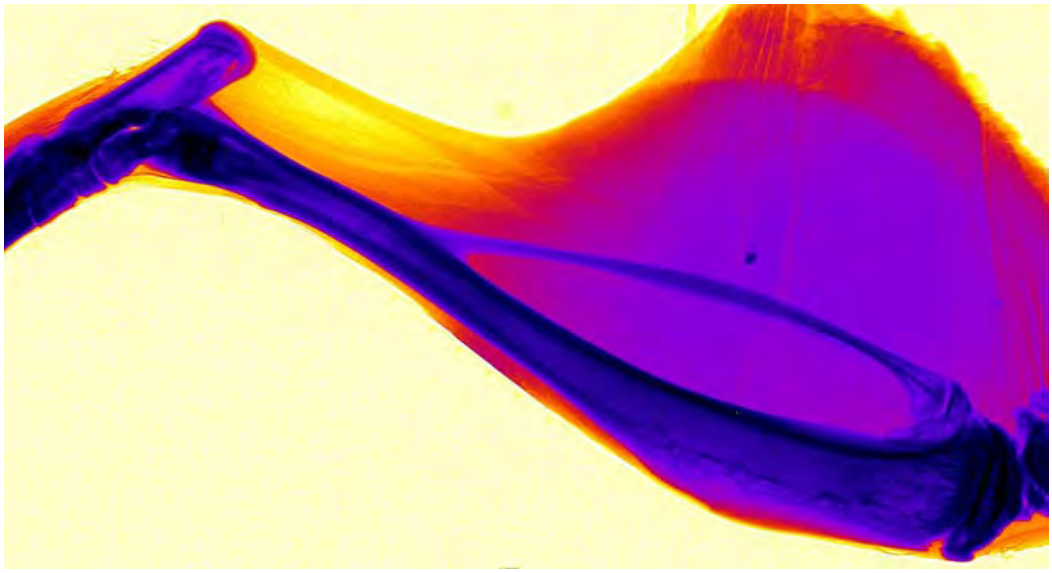

Figure 1: X-ray image of a section of a mouse hind leg. The exposure time was 3 min.

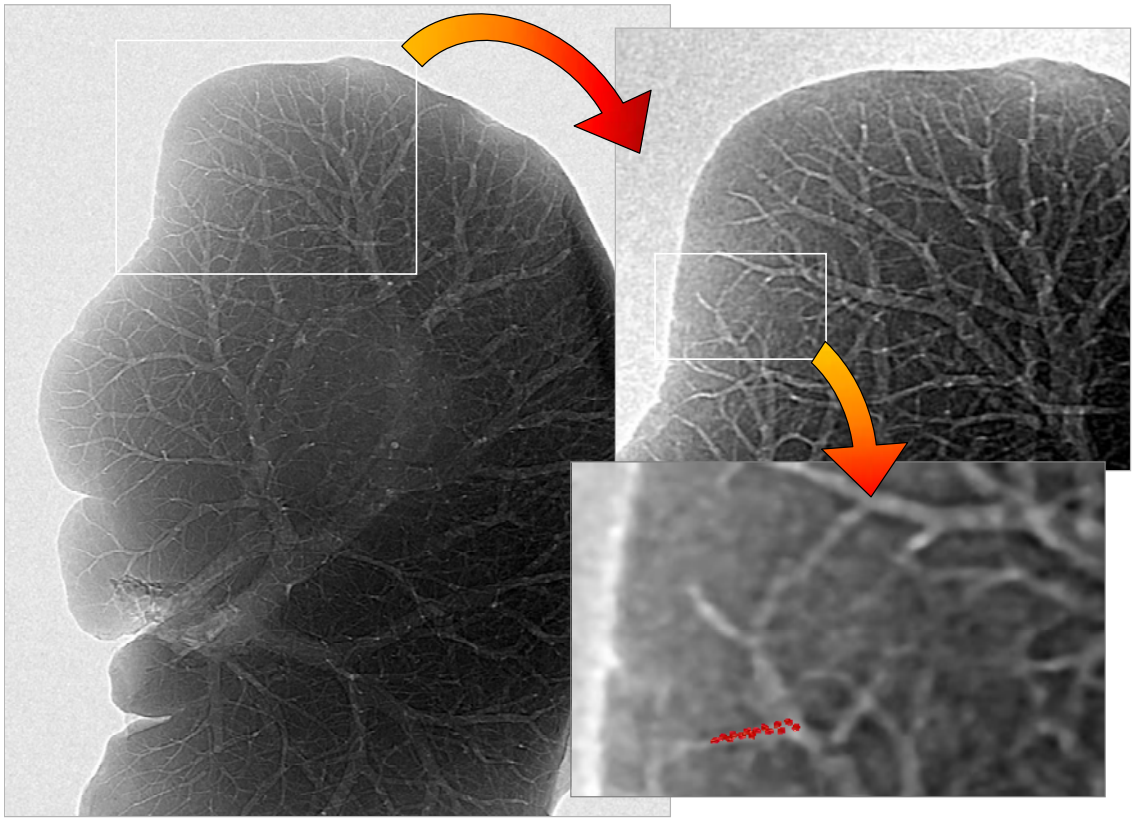

Figure 2: X-ray phase contrast images of a lobe of a mouse liver. The veins are “outlined” by dark phase lines. The image on the bottom right shows veins with a diameter of approximately 20 µm diameter. For scale comparison, pictures of blood cells are pasted into the image. The exposure time is 3 min / image.

Concerning the new preparative method using low density contrast agents, murine livers were extracted, placed in a 4% aqueous formaldehyde solution, and stored at room temperature for at least 72 hours to allow ample fixation time. In the case of murine livers, the fixation process together with dehydration results in filling of the venous network with air. Contrast agent injections into the portal veins of excised livers verified that the venous system is imaged and not the arterial system or bile ducts.

Figure 3 shows both the results of the sample preparation and the effect of post-processing images using a Fast Fourier Transform bandpass filtering algorithm. First note the contrast that has been obtained without the use of injected contrast agents. The bandpass filtering results in removal of gross absorption features caused by uneven thickness across the liver resulting in an enhancement of microvessel features. The arrows in Fig. 3(b) and (d) point out a microvessel that is 20 micron in diameter. For reference we note that an erythrocyte is approximately 7 micron in its longest dimension.

Figure 4 shows a time-lapse sequence of images taken as the fixed liver sample was dehydrated in ambient air. As the sample dried, evaporative water loss resulted in the introduction of air into the vascular network, providing both phase and absorption contrast in the image. The weight of the liver was recorded immediately after being withdrawn from the formaldehyde solution and was subsequently recorded at the time each image was made. The percentage indicated in each image is weight lost due to evaporation, relative to the initial weight. The images (b)-(e) are taken of the region outlined with a box in (a). Each image was taken with an exposure time of 80 s using 172 cm and 74 cm source-to-detector and source-to-object distances, respectively, yielding a magnification of 2.3. Completely dried liver samples lose approximately 80% of their original weight during drying and the resulting dried, hardened samples cannot be histologically analyzed. Using the liver preparation method described above, we observed no discernable difference in the appearance of vasculature after a liver has lost only 20% of its original weight. This finding implies that a liver prepared using this method may be radiographically imaged and subsequently histologically assessed, a potentially valuable synergy of two separate imaging modalities.

Aside from the high contrast provided by the tissue preparation method described here, two additional important aspects of the method are its ease of implementation and virtual certainty of success. The fixation and drying method, which results in a gaseous contrast agent is, in many ways, ideally suited to x-ray phase contrast imaging, which responds to spatial derivatives of the electron density. The favorable results shown here with healthy livers suggest the use of in-line, phase contrast imaging with gaseous contrasts agents for application to the study of vascular genesis in livers, and, pending the development of parallel fixation methods for other internal organs, to studies of other disease pathologies where imaging of vascular features is paramount.

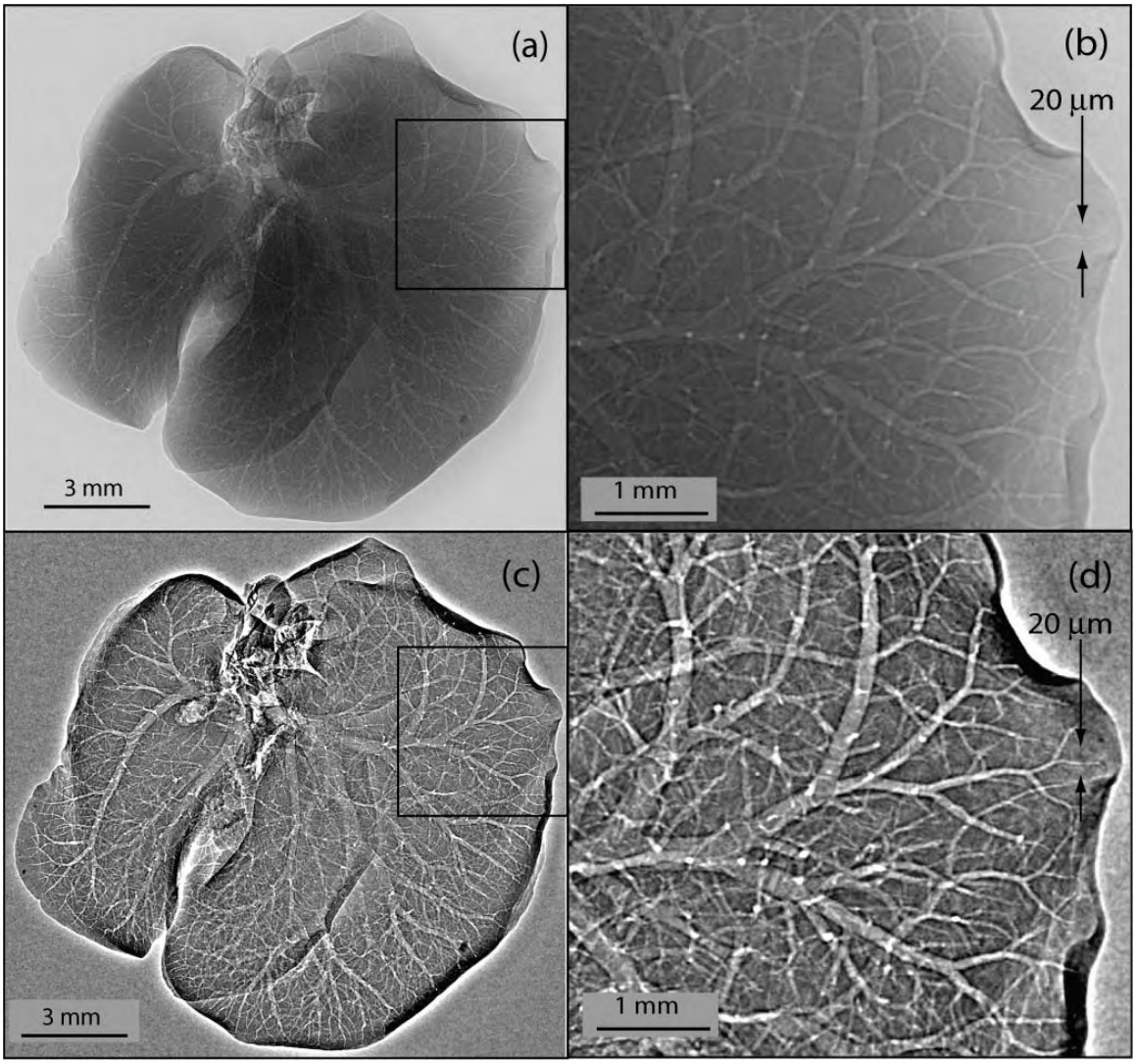

Figure 3 X-ray Images of fixed dried murine liver samples. (a) Unprocessed image of a whole liver sample; (b) a zoomed version of (a), denoted by the black box, highlighting a 20 micron diameter microvessel; (c) image of whole liver sample in (a) that has been processed with a 2-100 pixel spatial frequency bandpass filter algorithm, corresponding to 12 micron – 600 micron in the sample and (d) a zoomed version of the processed liver image from (c), denoted by the black box. The bright spots are found to be vessels pointing out of the plane of the image. The images were taken with an 80 second exposure with 200 cm and 80 cm source-to-detector and source-to-object distances, respectively, yielding a magnification of 2.5. The whole liver image has dimensions of 2588 x 2380 pixels, which, given the magnification, represents a 15.5 mm x 14.3 mm region of the liver.

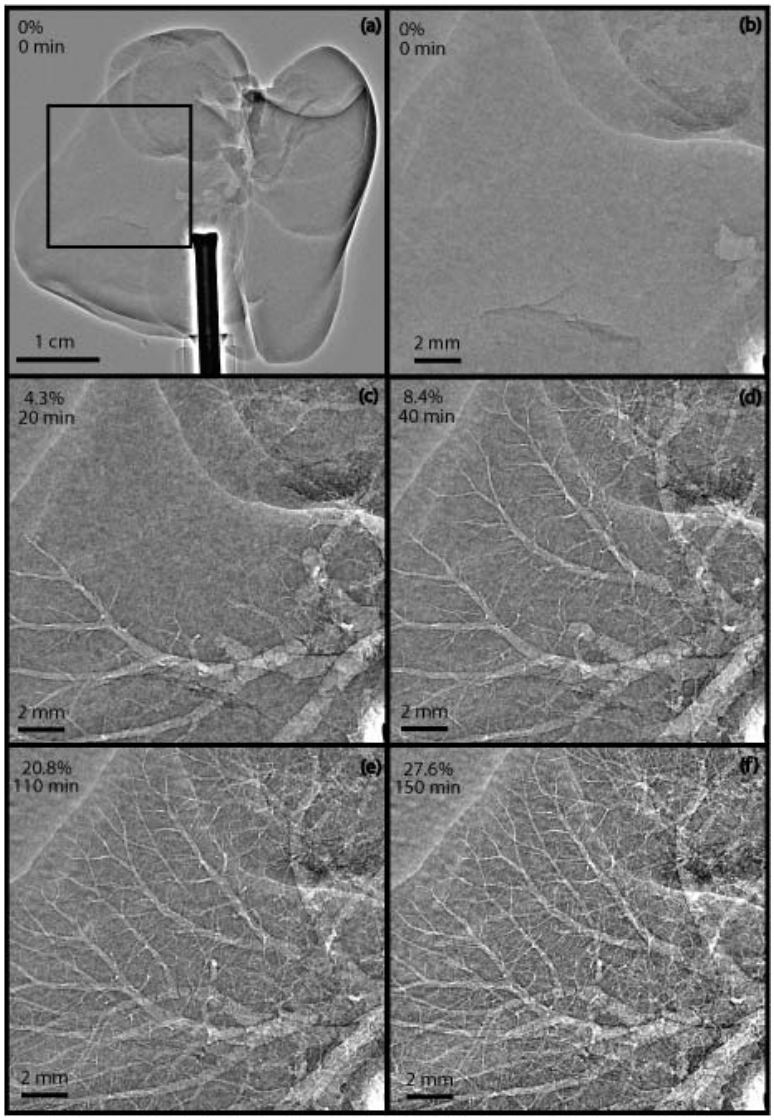

Figure 4: A time-lapse image sequence of a liver sample. The liver was mounted on a section of stainless steel tubing attached to precision scales. X-ray images of the liver were then taken at intervals of 20, 40, 110 and 120 min. as the liver dried in the ambient air. Figs b-f are 1316 x 1932 pixel regions of the liver image, which, given the magnification, represents an 8.6 mm x 12.6 mm region of the liver. The images were spatially filtered in the 2-100 pixel range. The ambient room conditions were 24 ^{o}C and 57% humidity.

Reportable Outcomes

1. "X-ray Phase Contrast Imaging: Transmission Functions Separable in Cartesian Coordinates", with G. Cao, T. Hamilton, and C. Rose-Petruck *J. Opt. Soc. Am. A.*, **24**, 1201-1208 (2007)

2. Photothermal Modification of X-ray Phase Contrast Images" C. Laperle, G. Cao, T. J. Hamilton C. Rose-Petruck, and G. J. Diebold, Progress in Biomedical

Optics and Imaging, Photons Plus Ultrasound: Imaging and Sensing 2007, **8** 64371N, (2007).

3. X-ray Phase Contrast Imaging: Transmission Functions Separable in Cylindrical Coordinates", Guohua Cao, Theron Hamilton, Christopher M. Laperle, Christoph Rose-Petruck, and Gerald J. Diebold, (Submitted to J. Appl. Phys.)

4. X-ray Elastography: Modification of X-ray Phase Contrast Images using Ultrasonic Radiation Pressure", Theron J. Hamilton, Claude Bailat, Stephan Gehring, Christopher M. Laperle, Jack Wands, Christoph Rose-Petruck, and Gerald J. Diebold accepted for publication *J. Appl. Phys*.

5. Propagation based differential phase contrast imaging and tomography of murine tissue with a laser plasma x-ray source", Xiaodi Li, Brian Ahr, Christopher M. Laperle, Philip Wintermeyer, Daxin Shi, Mark Anastasio, Jack Wands, Gerald J. Diebold*, and Christoph Rose-Petruck, Appl. Phys. Lett 91, 173901 (2007) also *Virtual Journal of Biological Physics Research*.

Conclusions

We have shown the feasibility of using a laser plasma x-ray source for high resolution imaging of biological samples. Although the inherently high time resolution of the laser x-ray source is evident, we have not used its time resolved capability. We have introduced a new method for fixing tissue that uses low electron density substances (air) as the contrast agent as opposed to the conventional high electron density contrast agents such as Ba and I compounds. The method is uniquely suited to phase contrast imaging, providing high contrast and permitting visualization of vascular features at the scale of several microns.

References

1. Born, M. & Wolf, E. *Principles of Optics* (ed. Press, P.) (Pergamon Press, Oxford, England, 1980).
2. Cowley, J. M. (ed.) *Electron Diffraction* (Kluwer Academic Publishers, Dordrecht, 1991).
3. Cowley, J. M. *Diffraction Physics* (North Holland Physics Publishing, a division of Elsevier Science Publishers B.V., Amsterdam, 1984).
4. Wilkins, S. W., Gureyev, T. E., Gao, D., Pogany, A. & Stevenson, A. W. Phase-contrast imaging using polychromatic hard x-rays. *Nature* **384**, 335-338 (1996).
5. Fulvia Arfelli, V. B., Alberto Bravin, Giovanni Cantatore, Edoardo Castelli et al. Mammography with Synchrotron Radiation: Phase Detection Techniques. *Radiology* **215**, 286-293 (2000).
6. Momose, A. Demonstration of phase-contrast x-ray computed tomography using an x-ray interferometer. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **352**, 622-8 (1995).
7. Pogany, A., Gao, D. & Wilkins, S. W. Contrast and resolution in imaging with microfocus x-ray source. *Rev. Sci. Instr.* **68**, 2774 (1997).
8. Krol, A. et al. Laser-based microfocused x-ray source for mammography: Feasibility study. *Medical Physics* **24**, 725-732 (1997).
9. Krol, A., Kieffer, J. C. & Forster, E. Laser-driven x-ray source for diagnostic radiology. *Proceedings of SPIE-The International Society for Optical Engineering* **3157**, 156-163 (1997).
10. Beckmann, F., Bonse, U., Busch, F. & Gunnewig, O. X-ray microtomography (micro CT) using phase contrast for the investigation of organic matter. *Journal of Computer Assisted Tomography* **21**, 539-53 (1997).